OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Stool DNA 96 Kit

M4016-00	1 x 96 preps
M4016-01	4 x 96 preps

November 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® Stool DNA 96 Kit

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Introduction

The Mag-Bind® Stool DNA 96 Kit allows rapid and reliable isolation of high-quality genomic DNA from stool samples which isolates both pathogen and host DNA. Up to 300 µL stool samples can be processed in less than 45 minutes. The system allows for automation after sample lysis via Thermo KingFisher™ Flex, Applied Biosystems® MagMAX™, Qiagen BioSprint, or other liquid handling instruments. Two protocols are available depending on the amount of inhibition and downstream sensitivity. It is recommended to begin with the standard protocol and if issues occur use the inhibitor rich sample protocol.

The system combines the Mag-Bind® technology with HTR Reagent to eliminate PCR inhibiting compounds such as humic acid from stool samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the Mag-Bind® Stool DNA 96 Kit for the first time, please read this booklet to become familiar with the procedure. Stool sample is homogenized and then treated in a specially formulated buffer. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-freeze step. Contaminants are further removed by extraction steps. Binding conditions are then adjusted and the sample is bound to magnetic beads. Four rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Kit Contents

Product Number	M4016-00	M4016-01
Purifications	1 x 96 preps	4 x 96 preps
E-Z 96 Disruptor Plates with Caps	1	4
Mag-Bind® Particles CND	2.2 mL	4.4 mL
HTR Reagent	12 mL	45 mL
SLX-Mlus Buffer	60 mL	240 mL
DS Buffer	5 mL	45 mL
SP2 Buffer	12 mL	30 mL
XP2 Buffer	60 mL	240 mL
VHB Buffer	22 mL	88 mL
SPM Wash Buffer	30 mL	4 x 30 mL
Elution Buffer	15 mL	50 mL
Proteinase K Solution	2.2 mL	8.8 mL
RNase A	220 µL	880 µL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Stool DNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CND, RNase A, and HTR Reagent must be stored at 2-8°C. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M4016-00	70 mL
M4016-01	70 mL per bottle

2. Dilute VHB Buffer with 100% ethanol follows and store at room temperature.

Kit	100% Ethanol to be Added
M4016-00	28 mL
M4016-01	112 mL

Mag-Bind® Stool DNA 96 Kit - Standard Protocol

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge capable of at least 4,000 x *g* and 4°C
- Magnetic Separation Device (Recommended Cat# AlpAqua® 96S A001322)
- Incubator capable of 70°C
- 96-well plates with a capacity of 1200 µL (ABgene AB-1127) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- 100% ethanol
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Prepare DNA Wash Buffer and VHB Buffer according to the instructions in the Preparing Reagents section on Page 4
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C

1. Briefly spin the E-Z 96 Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate and save the caps for use in Step 3.

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 200 µL can be recovered after Step 4.

5. Incubate at 70°C for 10 minutes. Briefly vortex the plate once during incubation.

6. Centrifuge at 4,000-6,000 x *g* for 10 minutes at room temperature.

7. Transfer 300 µL supernatant to a 96-well plate compatible with the Magnetic Separation Device used.

8. Add 600 µL XP2 Buffer and 20 µL Mag-Bind® Particles CND.

   Note: Mag-Bind® Particles CND and XP2 Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

9. Let sit at room temperature for 5 minutes.

10. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

12. Remove the plate containing the Mag-Bind® Particles CND from the Magnetic Separation Device.

13. Add 400 µL VHB Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

14. Let sit at room temperature for 2 minutes.

15. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

17. Remove the plate containing the Mag-Bind® Particles CND from the Magnetic Separation Device.

18. Add 400 µL SPM Wash Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

19. Let sit at room temperature for 2 minutes.

20. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

22. Repeat Steps 17-21 for a second SPM Wash Buffer wash step.

23. Leave the plate on the Magnetic Separation Device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.

24. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

25. Let sit at room temperature for 5 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

27. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

Mag-Bind® Stool DNA 96 Kit - Inhibitor Rich Protocol

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge capable of at least 4,000 x *g* and 4°C
- Magnetic Separation Device (Recommended Cat# AlpAqua® 96S A001322)
- Incubator capable of 70°C
- 96-well plates with a capacity of 1200 µL (ABgene AB-1127) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- Ice bucket
- Sealing film
- 100% ethanol
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Prepare DNA Wash Buffer and VHB Buffer according to the instructions in the Preparing Reagents section on Page 4
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice bucket
- Cool a centrifuge to 4°C

1. Briefly spin the E-Z 96 Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate and save the caps for use in Step 3.

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 200 µL can be recovered after Step 4.

5. Remove and save the caps.

6. Add 30 µL DS Buffer and 2 µL RNase A. Seal the plate with the caps.

7. Vortex to mix thoroughly.

8. Incubate at 70°C for 10 minutes. Briefly vortex the tubes once during incubation.

9. Centrifuge at 4,000-6,000 x *g* for 10 minutes at room temperature.

10. Transfer 300 µL supernatant to a new 96-well plate (not provided).

11. Add 100 µL SP2 Buffer and 100 µL HTR Reagent. Seal the plate with sealing film. Vortex to mix thoroughly.

    Note: Completely resuspend the HTR Reagent by shaking the bottle before use.

12. Let sit on ice for 5 minutes.

13. Centrifuge at 4,000-6,000 x *g* for 10 minutes at 4°C.

14. Transfer 300 µL supernatant to a new 96-well plate compatible with the Magnetic Separation Device used.

15. Add 600 µL XP2 Buffer and 20 µL Mag-Bind® Particles CND.

    Note: Mag-Bind® Particles CND and XP2 Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

16. Let sit at room temperature for 5 minutes.

17. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

18. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

19. Remove the plate containing the Mag-Bind® Particles CND from the Magnetic Separation Device.

20. Add 400 µL VHB Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

21. Let sit at room temperature for 5 minutes.

22. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

24. Remove the plate containing the Mag-Bind® Particles CND from the Magnetic Separation Device.

25. Add 400 µL SPM Wash Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

26. Let sit at room temperature for 2 minutes.

27. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

28. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

29. Repeat Steps 24-28 for a second SPM Wash Buffer wash step.

30. Leave the plate on the Magnetic Separation Device for 5-10 minutes to air dry the Mag-Bind® Particles CND. Remove any residual liquid with a pipettor.

31. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CND by vortexing or pipetting up and down 20 times.

32. Let sit at room temperature for 5 minutes.

33. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

34. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

Mag-Bind® Stool DNA 96 Kit - KingFisher™ Flex, KingFisher™ 96, Applied Biosystems MagMAX™, Qiagen Biosprint Protocol

Contact technical support at Omega Bio-tek for instrument files for your instrument.

Materials and Equipment to be Supplied by User:

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- Kingfisher Deepwell plates
- 96-well Kingfisher microplates for DNA storage
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- Refrigerated centrifuge capable of at least 4,000 x *g* and 4°C
- Thermo KingFisher™ Flex/Applied Biosystems MagMAX™/Qiagen Biosprint
- Incubator capable of 70°C
- KingFisher™ Deep Well plates
- KingFisher™ 96 KF microplate (200 µL) for DNA storage
- Vortexer
- Ice bucket
- 100% ethanol
- Nuclease-free water
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Set an incubator to 70°C

1. Briefly spin the E-Z 96 Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate and save the caps for use in Step 3.

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 200 µL can be recovered after Step 4.

5. Incubate at 70°C for 10 minutes. Briefly vortex the tubes once during incubation.

6. Centrifuge at 4,000-6,000 x *g* for 10 minutes at room temperature.

7. Transfer 300 µL supernatant to a KingFisher™ 96 Deep Well plate.

8. Add 600 µL XP2 Buffer and 20 µL Mag-Bind® Particles CND. ***This is the Lysate Plate.***

   Note: Mag-Bind® Particles CND and XP2 Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

9. Prepare the remaining plates as follows. The Lysate Plate was prepared in Steps 1-8.

Plate Type	Name	Contents	Volume
Deep Well	Lysate	Sample SLX-Mlus Buffer Proteinase K Solution XP2 Buffer Mag Bind® Particles CND	300 µL 300 µL 20 µL 600 µL 20 µL
Deep Well	VHB	VHB Buffer	400 µL
Deep Well	SPM 1	SPM Wash Buffer	400 µL
Deep Well	SPM 2	SPM Wash Buffer	400 µL
Deep Well	Water	Nuclease-free Water	500 µL
Microplate	Elution	Elution Buffer	100 µL
Microplate	Tip Pick Up	Magnetic Tip Comb	
Empty	Empty	Empty	

10. Press start on the instrument protocol and load the plates according to the prompts.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
A_{260}/A_{230} ratio is low	Inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix the sample with HTR Reagent thoroughly. Add 100 µL to cleared supernatant. Mix by vortexing. Let sit for two minutes. Centrifuge at 4,000 x *g* for 10 minutes and transfer cleared supernatant to next step. Do not reuse SP2 Buffer.
	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with VHB Buffer. • Wash the Mag-Bind® Particles CND with ethanol.
A_{260}/A_{280} ratio is high	RNA contamination	Be sure to treat the sample with RNase A according to the protocol.
Low DNA Yield or no DNA Yield	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample with SLX-Mlus Buffer thoroughly. Use a commercial homogenizer if possible.
	DNA washed off.	Make sure VHB Buffer and SPM Wash Buffer are mixed with ethanol.
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA elute used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
Problems in downstream applications	Inhibitory substance in the eluted DNA.	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary
	Ethanol residue in the elute	Extend the dry time of the Mag-Bind® Particles CND to 15 minutes before elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
96-well Racked Microtubes (1.2 mL), 10 rks/pk, 10 pk/cs	SSI-1760-00
96-well Racked Microtubes (1.2 mL), Sterile, 10 rks/pk, 10 pk/cs	SSI-1760-S0
Caps for Racked Microtube Caps, 8-Strip Caps, 125 strips/pk, 10 pk/cs	SSI-1702-00
Caps for Racked Microtube Caps, 8-Strip Caps, Sterile, 125 strips/pk, 10 pk/cs	SSI-1702-S0
1.2 mL Round-well Plate, 10/pk	SSI-1780-00
96-well Microplate (300 µL), 5/pk	EZ9603-01
96-well Microplate (300 µL), 25/pk	EZ9603-02
E-Z 96 Magnetic Separation Device	MSD-01B

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